Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk

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Genetic variations in DNA repair may impact repair functions,
DNA damage and breast cancer risk. Using data/samples col-
lected from the first 752 Caucasians and 141 African-Americans
in an ongoing case–control study, we examined the association
between breast cancer risk and 18 non-synonymous single-
nucleotide polymorphisms (nsSNPs) in four DNA repair
pathways—(i) base excision repair: *ADPRT V762A, APE1 D148E,
XRCC1 R194W/R280H/R399Q* and *POLD1 R119H*; (ii) nucleotide
excision repair: *ERCC2 D312N/K751Q, ERCC4 R415Q, ERCC5
D1104H* and *XPC A499V/K939Q*; (iii) mismatch repair: *MLH1
I219V, MSH3 R940Q/T1036A* and *MSH6 G39E* and (iv) double-
strand break repair: *NBS1 E185Q* and *XRCC3 T241M*. In Cau-
casians, breast cancer risk was significantly associated with
ADPRT 762VV [odds ratio (OR) = 1.45; 95% confidence interval
(CI) = 1.03, 2.03], *APE1 148DD* (OR = 1.44; 95% CI = 1.03,
2.00), *MLH1 219II/IV* (OR = 1.87; 95% CI = 1.11, 3.16) and
ERCC4 415QQ (OR = 8.64; 95% CI = 1.04, 72.02) genotypes.
With a limited sample size, we did not observe any significant
association in African-Americans. However, there were signifi-
cant trends in breast cancer risk with increasing numbers of risk
genotypes for *ADPRT 762VV, APE1 148DD, ERCC4 415RQ/QQ*
and *MLH1 219II/IV* ($P_{trend} < 0.001$) in Caucasians and *ADPRT
762VA, ERCC2 751KQ/QQ* and *NBS1 185EQ/QQ* in African-
Americans ($P_{trend} = 0.006$), respectively. Our results suggest that
combined nsSNPs in multiple DNA repair pathways may contribute
to breast cancer risk and larger studies are warranted to further
evaluate polygenic models of DNA repair in breast cancer risk.

Introduction

Breast cancer is the most common neoplasm and the second leading
cause of cancer death in American women. In 2008, it is estimated
that ∼182 460 and 40 480 American women will be diagnosed with
and die from breast cancer, respectively (1). Interindividual variations
in DNA damage and repair have been associated with an increased
risk of breast cancer (2,3). Rare germ line mutations in DNA damage
response genes, such as *BRCA1, BRCA2, ATM, FANC* and *CHEK2*,
are associated with breast cancer susceptibility and highlight the im-
portance of DNA damage/repair in the development of the disease (4).
To what extent common genetic variations in DNA repair genes con-
tribute to breast cancer risk, however, remains unclear. Given the
complexity of breast cancer etiology and interplay of different DNA
repair pathways in breast cancer, this study was designed to investi-

gate the association between breast cancer risk and common non-
synonymous single-nucleotide polymorphisms (nsSNPs) in four re-
pair pathways, including base excision repair (BER), nucleotide ex-
cision repair (NER), mismatch repair (MMR) and double-strand break
repair (DSBR). Efficient DNA repair capacity is essential in minimiz-
ing the accumulation of DNA damage, which may contribute to the
initiation of aberrant cell growth and human carcinogenesis develop-
ment. Reduced DNA repair capacity and elevated DNA damage in
breast cancer cases and in women with a family history (FH) of breast
cancer have been reported (5–7).

The BER pathway removes DNA damage caused by ionizing radi-
ation, reactive oxidative species and methylating agents. ADPRT/
PARP-1 is a vital member of the BER pathway; it senses DNA strand
breaks and initiates DNA damage signaling (8). Lower oligonucleo-
tide-induced ADPRT/PARP-1 activity has been reported in postmen-
opausal women at increased risk for breast cancer (9). APE1 is the
rate-limiting enzyme critical for single-nucleotide BER (10). XRCC1
has no known enzymatic activity, but it can interact and stimulate
enzymatic activities of other BER proteins (11). POLD1 has 3′-5′-
exonuclease activity that removes DNA lesions in close proximity to
ionizing radiation-induced DNA single-strand breaks (12).

NER plays a critical role in repairing various forms of DNA damage:
bulky adducts generated from genotoxic compounds, ultraviolet-
induced photo lesions and intrastrand cross-links (13). Acting as a 5′
DNA helicase, ERCC2/XPD is part of the basal transcription factor IIH,
a multiprotein complex in NER and transcription. ERCC4/XPF func-
tions as a 5′-endonuclease and forms a tight complex with ERCC1 in
NER and it has been implicated in homologous recombination (HR)
and interstrand cross-link repair (14). ERCC5/XPG protein functions as
one of the two endonucleases making dual incisions in NER; it is re-
quired for formation of the fully opened DNA conformation (15). XPC
plays a critical role in DNA damage recognition (15).

MMR is a highly conserved repair pathway that functions in im-
proving replication fidelity by correcting replication-associated base–
base and insertion/deletion mispairs (16). MMR also suppresses HR
and plays a role in DNA damage signaling (16). High-frequency
microsatellite instability is detected more frequently in bilateral but
not in unilateral breast cancers (17). Losses of heterozygosity and/or
microsatellite instability were detected in 83% of the skin samples
from breast cancer patients, which suggest a potential role of MMR in
breast cancer susceptibility (18). In *Saccharomyces cerevisiae*,
MSH2–MSH3 and MSH2–MSH6 function in mismatch recogni-
tion, and MLH1–PMS1 is the primary MLH heterodimer in post-
replicative MMR (19).

Double-strand breaks may result in cell death or a wide variety of
genetic alterations, including large- or small-scale deletions, loss of
heterozygosity, translocations and chromosome loss (20). Double-
strand breaks are repaired by at least two major repair pathways,
HR and non-homologous end joining. Five proteins (RAD51B,
RAD51C, RAD51D, XRCC2 and XRCC3) that share homology with
RAD51 recombinase are important for HR; the RAD51C–XRCC3-
associated Holliday junction resolvase complex may play an essential
role in the resolution of recombination intermediates (21). A number
of proteins influence both pathways, including the MRE11–RAD50–
NBS1 complex, BRCA1, histone H2AX, PARP-1, RAD18, DNA-
dependent protein kinase catalytic subunit and ATM. Breast cancer
risk is associated with the genes encoding the DNA DSBR MRE11–
RAD50–NBS1 complex and BRCA1–CtIP–MRE11–RAD50–NBS1
complex (22,23).

Different DNA repair pathways play vital roles in preserving ge-
nome stability and genetic variations in multiple repair pathways may
result in elevated breast cancer risk. Based on the assumption that
nsSNPs lead to amino acid substitutions and may result in altered
function, we hypothesize that nsSNPs from different repair pathways
have additive/multiplicative effects on breast cancer risk. Therefore,

this study evaluated the association between breast cancer risk and 18 nsSNPs in four DNA repair pathways—(i) BER: *ADPRT V762A* (rs1136410), *APE1 D148E* (rs3136820), *XRCC1 R194W* (rs1799782)/*R280H* (rs25489)/*R399Q* (rs25487) and *POLD1 R119H* (rs1726801); (ii) DSBR: *NBS1 E185Q* (rs1805794) and *XRCC3 T241M* (rs861539); (iii) MMR: *MLH1 I219V* (rs1799977), *MSH3 R940Q* (rs184967)/*T1036A* (rs26279) and *MSH6 G39E* (rs1042821) and (iv) NER: *ERCC2 D312N* (rs1799793)/*K751Q* (rs13181), *ERCC4 R415Q* (rs1800067), *ERCC5 D1104H* (rs17655) and *XPC A499V* (rs2228000)/*K939Q* (rs2228001).

Materials and methods

The case–control study design has been described previously (24). Study participants have been recruited at Wake Forest University Health Sciences since November 1998 (samples collected up to December 2004 were used for this study). Genotype data were available from 752 Caucasians (416 controls and 336 cases) and 141 African-Americans (78 controls and 63 cases). Newly diagnosed breast cancer cases, prior to any therapy, were enrolled at the Wake Forest University Breast Care Center. Histopathology and medical records were reviewed to confirm diagnosis. Controls were frequency matched to cases on age/race and recruited from the clinic population receiving routine mammography at the Breast Screening and Diagnostic Center. Eligibility criteria for controls included normal mammography results and no prior cancer history. Study participants reviewed a brief description of the protocol with a research co-ordinator and provided their signed, informed consent, as approved by the medical center's institutional review board. Whole blood (20 ml) was collected from enrolled subjects and processed within 2 h after phlebotomy. Every study participant completed a self-administered baseline questionnaire, which included information on demographics, reproductive history, medical conditions and FH of cancer. Positive FH of breast cancer was defined as a woman with mother and/or sister with breast cancer. Ever smoking history was defined as lifetime smoking history of at least 100 cigarettes.

Genotyping analysis

Genomic DNA was extracted from frozen whole blood using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA). The genetic polymorphisms of interest were selected based on three criteria: (i) the single-nucleotide polymorphism (SNP) resulted in an amino acid substitution; (ii) the variant allele frequency was approximately ≥5% in the general population and (iii) sequence information was available for accurate assay development. The MassARRAY system (Sequenom, San Diego, CA) was used to determine genotypes. Sequences of forward, reverse and extension primers for the DNA repair nsSNPs are listed in supplementary Table I (available at *Carcinogenesis* Online). Genotyping was first completed on a panel of 90 DNA samples from Coriell Institute for Medical Research (Camden, NJ) and compared with the reported genotype data on two Web sites: http://www.ncbi.nlm.nih.gov and http://egp.gs.washington.edu. As part of the quality control protocol, four control samples were genotyped with 92 patient samples on each 96-well plate, and study cases and controls were loaded on each plate to minimize systematic bias. The average call rate was >95% for the genotype assay. The concordance rate for the quality control samples was 100% and the concordance rate for the Coriell samples ranged from 91 to 100%. For each genotype, there was a 100% concordance rate for the four internal control samples on each plate. Each nsSNP was also tested for Hardy–Weinberg equilibrium (HWE).

Statistical analyses

Student's *t*-tests and χ^2 tests were used to compare case and control demographic variables. Comparisons between case and control genotype and allele frequencies and departure from HWE were examined with χ^2 tests and Fisher's exact tests. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between DNA repair genetic variants and breast cancer risk, adjusting for established breast cancer risks and variables selected from forward stepwise entry: age, FH of breast cancer, smoking history, age at menarche, body mass index (BMI) and age at first live birth. Adjusted ORs, 95% CIs and *P*-values for effect modification were calculated for DNA repair genetic variants, stratified by age, FH of breast cancer and smoking history. Only Caucasians with complete information for all three variables were included in these evaluations. Statistical analyses were completed using SPSS v. 15.0 (SPSS, Chicago, IL). We performed false discovery rate (FDR) analysis using the SAS PROC MULTTEST to control for error rate related to multiple comparisons (SAS Institute, Cary, NC).

Association between breast cancer risk and haplotypes of *ERCC2*, *XRCC1*, *XPC* and *MSH3* genes was investigated using SAS version 9.1. The Expectation-Maximization algorithm was used to generate maximum likelihood estimates of haplotype frequencies based on the observed genotypes under the assumption of HWE. Each individual was assigned the probability of possessing a particular haplotype pair. Using the HAPLOTYPE procedure, the inferred haplotypes were created and logistic regression was performed to test the association between haplotypes and breast cancer risk. The following variables were adjusted in the multivariate analysis: age, FH of breast cancer, smoking history, age at menarche, age at first live birth and BMI.

Multivariate adaptive regression splines (MARS)-logit model was used to examine the data for high-order gene–gene interactions (25). The data were analyzed using MARS 2.0 software (Salford Systems, San Diego, CA). This technique utilizes MARS software to estimate spline-based models and to construct a logistic regression analysis in SPSS. All variables were included in the initial model, but were sequentially eliminated if determined to be non-significant contributors to the model. In MARS, we tested for a maximum of 40 basis functions, and each nsSNP was designated as a categorical predictor. This study utilized a forward entry method in the logistic regression models, and the final models were adjusted for age, FH of breast cancer, smoking history, age at menarche, age at first live birth and BMI. Bayesian information criterion was used to determine model fit. In addition, we also investigated polygenic models using logistic regression of combined genotypes of significant results in Caucasians or OR ≥2 in African-Americans. Only women with complete genotype information for all DNA repair genes were included. OR, 95% CI and P_{trend} were calculated.

Results

Characteristics of the study population are summarized in Table I. Distributions of race, smoking history, FH of breast cancer, age at menarche and age were similar between cases and controls. BMI was marginally higher in cases compared with controls ($P = 0.06$). More cases had first live birth at a younger age ($P = 0.04$). Genotype distributions in controls were consistent with HWE except for *ERCC5* (rs17655) ($P = 0.03$) and *MSH6* (rs1042821) ($P = 0.002$) in Caucasians and *MSH6* (rs1042821) ($P = 0.04$) in African-Americans. As high genotype concordance rate (>95%) was achieved, the marginal deviation for *ERCC5* and *MSH6* (African-Americans) and HWE deviation for *MSH6* (Caucasians) may be related to limited sample size. The distribution of these SNPs did not differ between cases and controls and was not included in the polygenic models. Previous reports indicated that multiple SNPs in four genes in this study have low linkage disequilibrium. For *XRCC1*, the $r^2 = 0$ between rs1799782 and rs25489, $r^2 = 0.04$ between rs1799782 and rs25487 and $r^2 = 0.03$ between rs25489 and rs25487 (26). Based on the HapMap data, the following has been observed—(i) *XPC*: $r^2 = 0.296$ and $r^2 = 0.022$ between rs2228000 and rs2228001; (ii) *ERCC2*: $r^2 = 0.56$ and $r^2 = 0.111$ between rs1799793 and rs13181 and (iii) *MSH3*: $r^2 = 0.36$ and $r^2 = 0.136$ between rs184967 and rs26279 in populations of European ancestry and African ancestry, respectively. Since many DNA repair genotypes and allele frequencies are different between Caucasians and African-Americans, all data analyses were stratified by race. The minor allele frequencies in controls were similar to those reported previously (27–32).

Table II summarizes the associations between six BER nsSNPs and breast cancer risk. In Caucasians, the *ADPRT 762VV* (rs1136410) was significantly associated with breast cancer risk (OR = 1.45; 95% CI = 1.03, 2.03, *VA/AA* as referent). *APE1 148DD* (rs3136820) carriers were at increased risk (OR = 1.44; 95% CI = 1.03, 2.00, *DE/EE* as referent). With a limited sample size of African-Americans, we did not observe any significant association. As shown in Table III, *ERCC4 415QQ* (rs1800067) genotype was associated with a significantly elevated breast cancer risk (OR = 8.64; 95% CI = 1.04, 72.02, *RR* as referent). As shown in Table IV, Caucasians with the *MLH1 219II/IV* (rs1799977) genotypes were at higher risk for breast cancer (OR = 1.87; 95% CI = 1.11, 3.16, *VV* as referent). None of the DSBR nsSNPs showed significant association with breast cancer risk.

We also considered three potential risk modifiers, age, FH and smoking history. As presented in Table V, our data suggest stronger associations with breast cancer risk in *ADPRT 762VV* (rs1136410) among those with age ≤60 (OR = 1.97; 95% CI = 1.21, 3.21) or without FH of breast cancer (OR = 1.54; 95% CI = 1.04, 2.28); *APE1 148DD* (rs3136820) among women with age >60 (OR = 1.76; 95%

Table I. Demographic characteristics of the study population

Characteristic	Categories	Control (*n* = 494)	Case (*n* = 399)	*P*-value
Age (years)	Mean ± SD	58.7 ± 11.8	57.4 ± 13.0	0.12
	≤50	153 (31.0%)	125 (31.3%)	0.22
	51–60	109 (22.1%)	110 (27.6%)	
	61–70	136 (27.5%)	95 (23.8%)	
	≥71	96 (19.4%)	69 (17.3%)	
Race	African-American	78 (15.8%)	63 (15.8%)	1.00
	Caucasian	416 (84.2%)	336 (84.2%)	
FH[a]	No	407 (82.4%)	320 (80.2%)	0.40
	Yes	87 (17.6%)	79 (19.8%)	
Smoking history[b]	No	282 (57.4%)	224 (57.6%)	0.96
	Yes	209 (42.6%)	165 (42.4%)	
	Missing	3	10	
Age at menarche	≤12	219 (44.9%)	173 (45.3%)	0.27
	13–14	215 (44.1%)	154 (40.3%)	
	≥15	54 (11.1%)	55 (14.4%)	
	Missing	6	17	
BMI	Mean ± SD	27.20 ± 5.97	27.99 ± 6.37	0.06
	Missing	3	10	
Age first live birth	≤24	240 (49.1%)	223 (57.6%)	0.04
	25–29	124 (25.4%)	77 (19.9%)	
	≥30 or nulliparous	125 (25.6%)	87 (22.5%)	
	Missing	5	12	

[a]First-degree relatives with breast cancer (mother and/or sister).
[b]Lifetime smoking history of at least 100 cigarettes.

Table II. BER nsSNPs and breast cancer risk by race

SNP/rs#	Genotype	Caucasian			African-American	
		Control/case	OR (95% CI)[a]	*P*-value	Control/case	OR (95% CI)[a]
ADPRT V762A (rs1136410)	VV	272/236	Referent		69/46	Referent
	VA	114/71	**0.69 (0.49, 0.98)**	0.04	3/6	4.63 (0.93, 23.07)
	AA	11/7	0.70 (0.26, 1.88)		0/0	NA
	VA/AA	125/78	Referent		3/6	Referent
	VV	272/236	**1.45 (1.03, 2.03)**	0.03	69/46	0.22 (0.04, 1.08)
APE1 D148E (rs3136820)	DD	104/103	Referent		30/23	Referent
	DE	209/140	**0.66 (0.46, 0.93)**	0.02	33/22	1.01 (0.43, 2.36)
	EE	92/76	0.79 (0.51, 1.23)		12/8	0.95 (0.30, 2.96)
	DE/EE	301/216	Referent		45/30	Referent
	DD	104/103	**1.44 (1.03, 2.00)**	0.03	30/23	1.01 (0.46, 2.21)
XRCC1 R194W (rs1799782)	RR	370/282	Referent		65/47	Referent
	RW	40/37	1.21 (0.74, 1.97)		10/5	0.44 (0.12, 1.67)
	WW	1/5	8.74 (0.97, 78.23)		0/1	NA
	RR/RW	410/319	Referent		75/52	Referent
	WW	1/5	8.56 (0.96, 76.53)		0/1	NA
XRCC1 R280H (rs25489)	RR	363/298	Referent		69/49	Referent
	RH	44/26	0.72 (0.43, 1.21)		6/4	0.66 (0.14, 3.05)
	HH	1/0	NA		0/0	NA
	RH/HH	45/26	Referent		6/4	Referent
	RR	363/298	1.42 (0.85, 2.38)		69/49	1.52 (0.33, 7.06)
XRCC1 R399Q (rs25487)	RR	179/135	Referent		58/38	Referent
	RQ	181/141	1.01 (0.74, 1.40)		15/13	1.13 (0.44, 2.91)
	QQ	46/36	0.93 (0.56, 1.54)		1/1	2.19 (0.09, 52.25)
	RR	179/135	Referent		58/38	Referent
	RQ/QQ	227/177	1.00 (0.73, 1.35)		16/14	1.18 (0.47, 2.96)
POLD1 R119H (rs1726801)	RR	345/268	Referent		38/23	Referent
	RH	48/44	1.05 (0.67, 1.67)		23/22	1.87 (0.77, 4.51)
	HH	4/3	1.14 (0.25, 5.24)		9/7	1.30 (0.35, 4.80)
	RR	345/268	Referent		38/23	Referent
	RH/HH	52/47	1.07 (0.69, 1.65)		32/29	1.72 (0.75, 3.91)

[a]Adjusted for age, FH of breast cancer, smoking history, age at menarche, age at first live birth and BMI.

CI = 1.08, 2.88), without FH (OR = 1.56; 95% CI = 1.08, 2.26), or never smokers (OR = 1.57; 95% CI = 1.03, 2.40); *MSH3 1036AA* (rs26279) among never smokers (OR = 2.34; 95% CI = 1.22, 4.50); as well as *MSH6 39GG* (rs1042821) among women with age ≤60 (OR = 2.08; 95% CI = 1.17, 3.72). However, the only significant interaction was observed between age and *MSH6* (rs1042821) (*P* = 0.002).

The MARS-logit technique was utilized to explore gene–gene interactions and breast cancer susceptibility. Using a smaller data

Table III. NER nsSNPs and breast cancer risk by race

SNP/rs#	Genotype	Caucasian			African-American	
		Control/case	OR (95% CI)[a]	P-value	Control/case	OR (95% CI)[a]
ERCC2 D312N (rs1799793)	DD	161/126	Referent		57/33	Referent
	DN	188/137	0.39 (0.67, 1.28)		16/14	1.42 (0.57, 3.54)
	NN	42/41	1.22 (0.74, 2.02)		1/2	11.01 (0.56, 214.97)
	DD	161/126	Referent		57/33	Referent
	DN/NN	230/178	0.98 (0.72, 1.34)		17/16	1.65 (0.68, 4.00)
ERCC2 K751Q (rs13181)	KK	144/117	Referent		48/25	Referent
	KQ	198/148	0.94 (0.67, 1.31)		19/23	2.39 (0.99, 5.73)
	QQ	57/49	1.11 (0.70, 1.76)		5/4	1.73 (0.38, 7.83)
	KK	144/117	Referent		48/25	Referent
	KQ/QQ	255/197	0.98 (0.71, 1.34)		24/27	2.24 (0.99, 5.07)
ERCC4 R415Q (rs1800067)	RR	358/278	Referent		73/51	Referent
	RQ	47/39	1.09 (0.69, 1.73)		2/2	1.92 (0.20, 18.90)
	QQ	1/7	**8.64 (1.04, 72.02)**	0.046	0/0	NA
	RR/RQ	405/317	Referent		75/53	Referent
	QQ	1/7	**8.54 (1.03, 71.78)**	0.047	0/0	NA
ERCC5 D1104H (rs17655)	DD	256/195	Referent		18/13	Referent
	DH	124/113	1.21 (0.87, 1.67)		37/32	0.98 (0.37, 2.59)
	HH	28/12	0.57 (0.28, 1.17)		20/7	0.41 (0.12, 1.41)
	DD	256/195	Referent		18/13	Referent
	DH/HH	152/125	1.09 (0.80, 1.48)		57/39	0.78 (0.31, 1.97)
XPC A499V (rs2228000)	AA	211/178	Referent		61/44	Referent
	AV	161/116	0.84 (0.61, 1.15)		14/7	0.62 (0.21, 1.88)
	VV	29/23	0.86 (0.47, 1.57)		0/1	NA
	AV/VV	190/139	Referent		14/8	Referent
	AA	211/178	1.19 (0.88, 1.61)		61/44	1.42 (0.49, 4.15)
XPC K939Q (rs2228001)	KK	162/124	Referent		43/28	Referent
	KQ	182/147	1.11 (0.80, 1.53)		26/23	1.75 (0.74, 4.13)
	QQ	62/50	1.04 (0.66, 1.62)		5/2	0.66 (0.10, 4.39)
	KK	162/124	Referent		43/28	Referent
	KQ/QQ	244/197	1.09 (0.80, 1.48)		31/25	1.55 (0.68, 3.54)

[a]Adjusted for age, FH of breast cancer, smoking history, age at menarche, age at first live birth and BMI.

set with complete genotype data (679 Caucasians and 111 African-Americans), no significant gene–gene interaction was identified. Previous studies have reported a higher susceptibility to cancer and cancer recurrence with increasing numbers of putative risk alleles (24,33). Polygenic models incorporating all DNA repair 'at-risk' genotypes were assessed (Table VI). There were significant trends in breast cancer risk with increasing numbers of risk genotypes for ADPRT (rs1136410), APE1 (rs3136820), ERCC4 (rs1800067) and MLH1 (rs1799977) ($P_{trend} < 0.001$) in Caucasians and ADPRT (rs1136410), ERCC2 (rs13181) and NBS1 (rs1805794) in African-Americans ($P_{trend} = 0.006$), respectively. In Caucasians, breast cancer was associated with combined two, three and four risk genotypes with OR of 1.73 (95% CI = 1.16, 2.58), 2.36 (95% CI = 1.48, 3.75) and 3.08 (95% CI = 1.05, 8.99), respectively. In African-Americans, breast cancer was associated with at least two risk genotypes (OR = 6.27; 95% CI = 1.71, 22.92). In addition, FDR was calculated, assuming 23 tests were performed; 18 single SNP association tests and 5 combined SNP tests. As compared with Caucasian women with zero or one risk genotypes, FDR indicated that Caucasian women with three risk genotypes were at significantly increased risk for breast cancer (FDR = 0.023, original P-value < 0.001). Caucasian women with two risk genotypes had a suggestive risk (FDR = 0.081, original P-value = 0.007), as compared with carriers of zero or one risk genotype. FDR did not suggest significant findings in African-Americans.

Our data suggested a borderline significance of three haplotypes and breast cancer risk. The first haplotype contained the G and A major alleles of ERCC2 gene at codons 312 ($G \rightarrow A$) and 751 ($A \rightarrow C$). This haplotype was found more frequently among controls (76.2%) than cases (66.8%) in African-Americans ($P = 0.05$). The second haplotype contained C, A and A alleles of XRCC1 gene at codons 194 ($C \rightarrow T$), 280 ($G \rightarrow A$) and 399 ($G \rightarrow A$). In Caucasians,

this uncommon haplotype was found only in controls (0.001%) but not in cases ($P = 0.06$). The third haplotype contained T, G and G alleles of XRCC1 gene at codons 194 ($C \rightarrow T$), 280 ($G \rightarrow A$) and 399 ($G \rightarrow A$). In Caucasians, this haplotype was more frequent in cases (6.7%) than in controls (5%) ($P = 0.07$).

Discussion

Using the candidate pathway approach, our current data suggest polygenic models of breast cancer risk. Although an individual DNA repair genotype may have a small effect, there was a combined effect of multiple genotypes from different pathways on breast cancer risk. Breast cancer has great genetic heterogeneity, most probably influenced by the contributions of combined variations in steroid hormone, metabolism, cell growth/apoptosis and DNA repair genes. The results from a multiethnic breast cancer study of 60 DNA repair genes showed that a variant in the FANCA gene (rs1061646) was significantly associated with breast cancer (34). In a recent genome-wide association study, several novel breast cancer susceptibility loci were identified (35). Genome-wide association studies may provide new targets for future research. Considering the multifactorial nature of breast cancer etiology, interactions among genetic, environmental exposures and host factors need to be considered simultaneously in order to adequately address breast cancer susceptibility (33).

In Caucasians, our current data suggest that breast cancer may be associated with ADPRT 762VV, APE1 148DD, ERCC4 415QQ and MLH1 219II/IV genotypes. Our current data on ADPRT 762VV genotype is inconsistent with two previous studies showing no association with breast cancer (36,37). Furthermore, our in vivo data and a recent in vitro study demonstrated that the ADPRT 762 A allele is associated with reduced enzyme activity (38,39). Therefore, the implication of our current findings is not clear. Considering the important roles that

Table IV. MMR/DSBR nsSNPs and breast cancer risk by race

SNP/rs#	Genotype	Caucasian			African-American	
		Control/case	OR (95% CI)[a]	P-value	Control/case	OR (95% CI)[a]
MLH1 I219V (rs1799977)	II	176/161	Referent		64/39	Referent
	IV	171/130	0.84 (0.61, 1.16)		9/11	2.20 (0.75, 6.41)
	VV	53/23	**0.49 (0.29, 0.85)**	0.01	0/1	NA
	VV	53/23	Referent		0/1	Referent
	II/IV	347/291	**1.87 (1.11, 3.16)**	0.02	73/50	NA
MSH3 R940Q (rs184967)	RR	288/230	Referent		56/35	Referent
	RQ	110/80	0.91 (0.64, 1.28)		18/16	1.44 (0.61, 3.43)
	QQ	10/15	1.73 (0.75, 3.99)		1/2	2.23 (0.17, 28.70)
	RR	288/230	Referent		56/35	Referent
	RQ/QQ	120/95	0.98 (0.71, 1.36)		19/18	1.50 (0.65, 3.45)
MSH3 T1036A (rs26279)	TT	198/154	Referent		33/21	Referent
	TA	175/123	0.94 (0.68, 1.31)		29/23	1.26 (0.51, 3.11)
	AA	36/44	1.45 (0.87, 2.43)		10/8	1.23 (0.35, 4.26)
	TT/TA	373/277	Referent		62/44	Referent
	AA	36/44	1.50 (0.92, 2.45)		10/8	1.10 (0.34, 3.52)
MSH6 G39E (rs1042821)	GG	337/271	Referent		58/40	Referent
	GE	58/41	0.83 (0.53, 1.31)		11/11	1.39 (0.50, 3.91)
	EE	9/7	0.82 (0.27, 2.52)		3/1	0.78 (0.07, 8.63)
	GE/EE	67/48	Referent		14/12	Referent
	GG	337/271	1.12 (0.74, 1.70)		58/40	1.11 (0.43, 2.86)
NBS1 E185Q (rs1805794)	EE	182/163	Referent		46/25	Referent
	EQ	176/127	0.79 (0.58, 1.09)		22/24	2.26 (0.97, 5.24)
	QQ	49/28	0.63 (0.37, 1.06)		6/4	1.36 (0.30, 6.09)
	EE	182/163	Referent		46/25	Referent
	EQ/QQ	225/155	0.76 (0.56, 1.03)		28/28	2.06 (0.93, 4.56)
XRCC3 T241M (rs861539)	TT	158/124	Referent		48/32	Referent
	TM	184/137	0.94 (0.68, 1.31)		20/19	1.98 (0.79, 4.95)
	MM	59/54	1.10 (0.71, 1.72)		5/1	0.24 (0.02, 2.40)
	TT	158/124	Referent		48/32	Referent
	TM/MM	243/191	0.98 (0.72, 1.34)		25/20	1.44 (0.62, 3.36)

[a]Adjusted for age, FH of breast cancer, smoking history, age at menarche, age at first live birth and BMI.

Table V. DNA repair nsSNPs and breast cancer risk by age, FH of breast cancer and smoking history (Caucasians only)

SNP	Genotype	Age[a]				FH of breast cancer[b]				Smoking history[c]			
		≤60		>60		No		Yes		Never		Ever	
		Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case
ADPRT V762A (rs1136410)	VA/AA	68	37	57	41	95	54	30	24	70	48	55	30
	VV	140	138	132	98	223	193	49	43	158	135	114	101
	OR (95% CI)	**1.97 (1.21, 3.21)***		1.07 (0.66, 1.74)		**1.54 (1.04, 2.28)***		1.25 (0.62, 2.55)		1.32 (0.84, 2.07)		1.63 (0.96, 2.79)	
APE1 D148E (rs3136820)	DE/EE	153	123	148	93	241	167	60	49	165	119	136	97
	DD	56	53	48	50	85	85	19	18	66	69	38	34
	OR (95% CI)	1.21 (0.76, 1.91)		**1.76 (1.08, 2.88)***		**1.56 (1.08, 2.26)***		1.08 (0.49, 2.41)		**1.57 (1.03, 2.40)***		1.28 (0.74, 2.22)	
MSH3 T1036A (rs26279)	TT/TA	190	152	183	125	300	219	73	58	217	161	156	116
	AA	22	27	14	17	30	34	6	10	17	29	19	15
	OR (95% CI)	1.40 (0.76, 2.60)		1.75 (0.82, 3.75)		1.46 (0.85, 2.48)		2.45 (0.79, 7.61)		**2.34 (1.22, 4.50)***		0.99 (0.47, 2.08)	
MSH6 G39E[d] (rs1042821)	GE/EE	46	21	21	27	56	40	11	8	36	23	31	25
	GG	161	157	176	114	270	212	67	59	195	165	142	106
	OR (95% CI)	**2.08 (1.17, 3.72)***		**0.53 (0.28, 0.99)***		1.12 (0.71, 1.77)		1.11 (0.40, 3.08)		1.24 (0.70, 2.21)		0.98 (0.54, 1.79)	

[a]Adjusted for FH of breast cancer (no/yes), age at menarche (≤12, 13–14 and ≥15), age at first live birth (≤24, 25–29, ≥30 or nulliparous), BMI (continuous) and smoking history (never/ever).
[b]Adjusted for age (continuous), age at menarche, age at first live birth, BMI and smoking history.
[c]Adjusted for age, FH of breast cancer, age at menarche, age at first live birth and BMI.
[d]A significant interaction between age and *MSH6 G39E* genotype ($P = 0.002$).
*P-value < 0.05.

ADPRT plays in DNA damage sensing and repair, its association with breast cancer risk warrants future research. Our current data on *APE1 148DD* genotype does not support the results from a previous study showing no association with breast cancer risk (37). In two of our previous studies with another study population, we demonstrated that *APE1* (rs3136820) and/or *XRCC1* (rs25487) may interact with FH and contribute to ionizing radiation hypersensitivity and susceptibility to breast cancer (5,40). Although *APE1* (rs3136820) SNP may not alter DNA binding or endonuclease activity, it may result in inefficient communication with other BER proteins (41). Protein–protein

Table VI. Polygenic models of breast cancer by race

Group	Total number of risk genotypes	Controls	%	Cases	%	OR (95% CI)[a]	P-value
Caucasian[b]	0–1	111	29.4	53	17.8	Referent	
	2	182	48.3	153	51.3	**1.73 (1.16, 2.58)**	0.007
	3	77	20.4	83	27.9	**2.36 (1.48, 3.75)**	<0.001
	4	7	1.9	9	3.0	**3.08 (1.05, 8.99)**	<0.001
						P_{trend} <0.001	
African-American[c]	0	24	35.8	9	17.6	Referent	
	1	34	50.7	26	51.0	2.27 (0.83, 6.24)	
	2+	9	13.4	16	31.4	**6.27 (1.71, 22.92)**	0.006
						P_{trend} = 0.006	

[a]Adjusted for age, FH of breast cancer, smoking history, age at menarche, age at first live birth and BMI.
[b]In Caucasians, risk genotypes *ADPRT 762VV* (rs1136410), *APE1 148DD* (rs3136820), *ERCC4 415RQ/QQ* (rs1800067) and *MLH1 219II/IV* (rs1799977) were included in the model.
[c]In African-Americans, risk genotypes *ADPRT 762VA* (rs1136410), *ERCC2 751KQ/QQ* (rs13181) and *NBS1 185EQ/QQ* (rs1805794) were included in the model.

interactions are essential to efficient BER, and thus *APE1 D148E* may influence overall co-ordination of BER activity.

Our data show that Caucasian *MLH1 219VV* genotype carriers had a decreased risk of breast cancer, which is similar to the findings in young-onset lung cancer patients (42). To the best of our knowledge, there was one published study reporting null association between *MLH1* (rs1799977) and breast cancer risk in Korean women (43). Although the *MLH1 I219V* polymorphism does not have an impact on enzyme function *in vitro* (44), it may be associated with childhood acute lymphoblastic leukemia susceptibility (45) and ulcerative colitis refractory to treatment with 6-mercaptopurine or azathioprine (46). Our current data support the previous observation that the *ERCC4 415QQ* genotype may be associated with breast cancer risk (24). Intriguingly, our finding is consistent with the results from a larger study conducted in North Carolina with 1133 controls and 1246 cases (31), but not in two other study populations in Maryland and New York (30,47). It is not clear whether there is a geographic difference in exposures or other factors that may impact genotype–cancer association. Furthermore, our data on *ERCC4 415QQ* as well as *XRCC1 194WW* need to be interpreted with caution since the risk association was calculated with only one control with the at-risk genotype.

With a limited sample size, we did not observe a significant association between breast cancer risk and individual nsSNP in African-Americans. However, three genotypes showed a suggestive association with breast cancer risk; and there was a significant trend in cancer risk with increasing numbers of risk genotypes for *ADPRT 762VA*, *ERCC2 751KQ/QQ* and *NBS1 185EQ/QQ* in African-Americans (P_{trend} = 0.006). The *ADPRT 762 A* allele has reduced enzyme activity (38,39) and potential association with breast cancer risk in African-Americans. The *ERCC2 D312N* SNP alone was not associated with breast cancer in African-Americans (31). In terms of the *NBS1 E185Q* SNP, our genotype distribution is very similar to another study in NC in Caucasians but not in African-Americans (48). Our data showed that a higher percentage of breast cancer cases has the *NBS1 185EQ/QQ* genotypes in African-Americans. With a limited sample size, this may be a chance finding and requires future validation.

Effect modification by age, FH and smoking history was evaluated. Although we found potential risk modification effects of age for *ADPRT* (rs1136410) or *APE1* (rs3136820), FH for *ADPRT* (rs1136410) or *APE1* (rs3136820) and smoking history for *APE1* (rs3136820) or *MSH3* (rs26279), the only significant interaction was between age and *MSH6* (rs1042821). The results from a previous study suggest that MSH6 and p53 deficiencies may interact to accelerate microsatellite instability and tumorigenesis (49). Young breast cancer cases are more probably to have *p53* mutations (25). Therefore, the *MSH6 39GG* genotype in combination with *p53* mutations may have a greater impact on young women. Further research is clearly needed to adequately assess how gene func-

tion–environment exposures modify disease susceptibility. Potential racial/ethnic-specific genotype–risk associations also suggest heterogeneity in breast cancer etiology, exposures, minor allele frequencies and susceptibility to environmental agents. Study inclusion of multiple genetic variants in polygenic models may enhance the understanding of genetic variations of DNA damage/repair and cancer risk (5,50). Although we did not observe gene–gene interactions using the MARS-logit models, our current data are compatible with a polygenic model that individual DNA repair genotype has a small effect on breast cancer risk. However, there were combined effects of DNA repair genotypes from different pathways on breast cancer risk.

Our study has several limitations. First, this study is part of our second phase of the genotyping effort from an ongoing breast cancer case–control study (820 cases and 859 controls as of 1 July 2008). Future studies with larger sample size will focus on a more comprehensive evaluation of DNA repair genotypes and functional phenotypes. With a limited number of African-American cancer cases seen in the clinic, we did not have adequate statistical power. Therefore, promising study results will need to be confirmed in larger studies of African-Americans. The major strengths of our overall study design are (i) hypothesis-driven DNA repair SNP selection and testing; (ii) adequate laboratory assay quality control; (iii) available cryopreserved lymphocytes for future functional assays to support genotype–risk association and (iv) both cases and controls were selected from a similar population. Our future studies will be strengthened by larger sample size.

In summary, our current data suggest that individual DNA repair genotype may have a small effect on breast cancer risk. However, there is a combined effect of DNA repair genotypes from different pathways on breast cancer risk. Although molecular and genetic epidemiologists now have the tools to comprehensively assess genetic susceptibility for cancer risk, including the genome-wide association and candidate pathway studies, we are facing the challenge of study designs for genetic investigation and the integration of gene–gene and gene–environment interactions in order to understand the complex mechanisms underlying breast cancer susceptibility. A comprehensive evaluation of DNA repair genetic variants and/or functional phenotypes in breast cancer risk may be necessary to identify susceptible populations.

Supplementary material

Supplementary Table I can be found at http://carcin.oxfordjournals.org/

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